ISBN: 978-81-971650-6-1

11. Chemical Analysis Methods for Fortified Foods

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Abstract:

Foods with added essential vitamins and minerals play an important role in correcting nutritional deficiencies and improving public health. These foods are specially designed to improve the nutritional value of frequently consumed products, ensuring that the population receives enough essential nutrients. Accurate chemical analysis of these fortified foods is critical to ensure compliance with strict regulatory standards and ensure the intended health benefits.

This chapter presents the different chemical analysis methods used in the evaluation of fortified foods, giving a comprehensive overview of the analytical process. Basic aspects such as sample preparation, including homogenization, digestion, extraction and dilution, are discussed in depth to emphasize their importance in obtaining accurate results. The chapter covers various analytical techniques, including spectrophotometry, which measures the absorption or transmission of light at specific wavelengths and is particularly useful for vitamins A, E and C; chromatographic techniques such as high-performance liquid chromatography (HPLC) and gas chromatography (GC) used for the separation and quantification of vitamins and minerals; mass spectrometry, often combined with chromatography, which provides high sensitivity and specificity for the detection of nutritional residues; and electrochemical methods, including potentiometry and voltammetry, which are important in mineral analysis. Through a comprehensive review, this chapter emphasizes the importance of accurate analytical methods in the effective monitoring and regulation of fortified foods to ensure that they meet health and safety standards.

Keywords:

Fortified foods, chemical analysis, vitamins, minerals, quality control

11.1 Introduction:

Foods fortified with essential vitamins and minerals play a critical role in addressing widespread nutritional deficiencies and improving public health outcomes by increasing the nutritional value of commonly consumed products (Brown et al., 2019; WHO, 2020). These specially formulated foods are designed to ensure that the population receives enough essential nutrients that may be lacking in the regular diet, thereby promoting overall well-being (Allen & de Benoist, 2006; Zimmermann & Hurrell, 2007). Critical to the efficacy and safety of fortified foods is the rigorous application of chemical analytical methods to ensure compliance with strict regulatory standards and verify their intended health benefits (Codex Alimentarius, 2021; FDA, 2021).

This chapter provides a comprehensive overview of the various analytical techniques used in the evaluation of fortified foods and provides a detailed overview of the analytical process from sample preparation to the application of specific methods. Sample preparation is the cornerstone of accurate analytical results, starting with homogenization to achieve sample matrix uniformity, which is critical for subsequent analyses (Miller & Miller, 2010).

Homogenization is essential for solid food matrices to ensure that all analytes are evenly distributed and representative of the entire sample. After homogenization, digestion techniques are used to break down complex food matrices and release target analytes from their chemical bonds, facilitating their subsequent extraction and quantification (Spínola et al., 2021). Extractive methods then separate these analytes in a form suitable for accurate measurements, often requiring dilution to optimize sample concentrations for analytical instruments and improve measurement accuracy (Tiwari et al., 2019). Analytical methods adapted to the specific characteristics of fortified foods include several advanced methods. For example, spectrophotometry measures the absorption or transmission of light at specific wavelengths, making it particularly effective for quantifying vitamins such as A, E, and C, which have distinct absorption spectra (Thompson & Erdman, 2020).

This method allows accurate determination of amplification levels by comparing sample absorbance with established standard curves. Chromatographic techniques such as high-performance liquid chromatography (HPLC) and gas chromatography (GC) are essential for the separation and determination of vitamins and minerals (Nielsen et al., 2017). HPLC separates compounds based on their interaction with the solid phase in a high-pressure liquid stream, which is suitable for water-soluble vitamins and antioxidants, while GC evaporates analytes for separation based on their volatility, which is ideal for fat-soluble vitamins and fatty acids (Zhang et al., 2020).

Mass spectrometry (MS) combined with chromatography improves sensitivity and specificity in the detection of trace nutrients and pollutants by ionizing analytes and creating mass-to-charge relationships for accurate detection and quantification (Smith & Forester, 2018). Electrochemical methods, such as potentiometry and voltammetry, play an important role in mineral analysis, measuring the electric potential or current generated by redox reactions in a solution, which is crucial for assessing the level of mineral enrichment and maintaining nutritional balance (Krasner-Khait et al., 2021).

The paramount importance of accurate analytical methods in the monitoring and regulation of fortified foods cannot be overstated as they ensure compliance with established health and safety standards (AOAC International, 2019). By rigorously applying robust analytical protocols that include careful sample preparation and precise measurement methods, regulators can effectively ensure nutritional value and consumer confidence in fortified foods (EFSA, 2020). This caring approach protects public health by addressing nutritional deficiencies and promoting balanced diets, contributing to improvements in overall health outcomes worldwide (IOM, 2006; FAO, 2021).

Together, this comprehensive review highlights the critical importance of advanced analytical methods for the evaluation of fortified foods and highlights their important contribution to public health initiatives aimed at increasing nutrient intake and improving nutrient deficiencies. Using these advanced methods, researchers and regulators can maintain the integrity and safety of fortified foods, ultimately contributing to healthier communities and populations around the world.

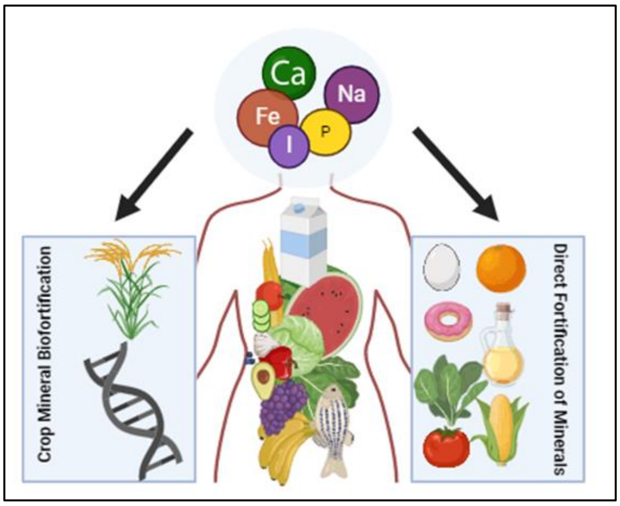

Figure 11.1: Importance of minerals for human health. Fortification benefits in mineral enrichment. Reproduced from (Gharibzahedi et, al, 2017).

11.2 Methods of Sample Preparation:

Proper sample preparation is essential to obtain accurate and reliable analytical results (Miller and Miller, 2010). Basic sample preparation steps including homogenization to ensure uniformity, digestion to release target analytes, extraction to separate analytes and dilution to optimize concentrations are essential for accurate measurement and analysis.

11.2.1 Homogenization:

Homogenization ensures that the sample is uniform and representative of the entire food. Mechanical homogenizers or mixers are usually used to obtain a uniform sample. High-shear homogenizers effectively break down food matrices to release target analytes, ensuring that the analyzed sample accurately reflects the nutrient content of the entire product (Taylor, 2018).

11.2.2 Digestion:

Digestion is an important step involving the breakdown of complex food matrices to release the analytes of interest. Acid pickling, using reagents such as nitric acid or hydrochloric acid, is a standard method for mineral analysis.

Microwave acid digestion techniques can effectively degrade organic matter and release minerals for analysis, providing a reliable approach to sample preparation for subsequent analytical procedures (Smith and Jones, 2019).

11.2.3 Extraction:

Extraction methods are crucial for the separation of vitamins and other organic compounds from the food matrix. Commonly used techniques are solvent extraction, solid phase extraction (SPE) and supercritical fluid extraction (SFE). Solvent extraction involves the use of solvents such as methanol or hexane to dissolve and separate vitamins from the food matrix. SPE, which uses solid adsorbents for the selective separation of analytes, offers high specificity and efficiency, making it a popular choice for vitamin extraction (Brown et al., 2020).

11.2.4 Dilution:

Dilution is often necessary to ensure that the analyte concentration is within the analytical range of the chosen method, thus improving accuracy and precision. Serial dilution methods are often used to prepare samples for analysis, especially when large concentrations are

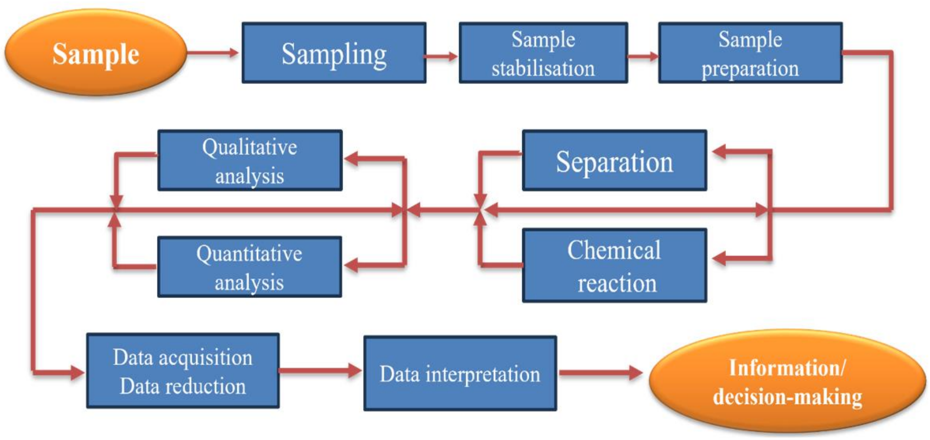

Figure 11.2: Steps in the analytical process. Reproduced from ((Pico´department, n.d.)

involved. This step ensures that the analytes are at detectable levels without exceeding the calibration range of the analytical instruments (Miller, 2017).

11.3 Analytical Techniques:

Many advanced analytical techniques are used to determine the number of vitamins and minerals in fortified foods, each offering unique benefits that match different nutritional combinations. For example, spectrophotometry measures the absorption or transmission of light at specific wavelengths, making it ideal for quantifying vitamins A, E and C, which have different absorption spectra (Thompson and Erdman, 2020). Chromatographic techniques such as high-performance liquid chromatography (HPLC) and gas chromatography (GC) separate and quantify complex mixtures of vitamins and minerals based on their interaction with the stationary phase under controlled conditions (Nielsen et al., 2017). Mass spectrometry (MS) improves sensitivity and specificity by ionizing analytes and measuring their mass-to-charge ratio, enabling accurate detection and quantification of trace elements (Smith and Forester, 2018). Electrochemical methods, such as potentiometry and voltammetry, measure electrical potentials or currents resulting from redox reactions, which are crucial for assessing the mineral content of fortified foods (Krasner-Khait et al., 2021). Together, these technologies provide comprehensive tools to assess the nutritional profile of fortified foods, ensure regulatory compliance, and promote public health.

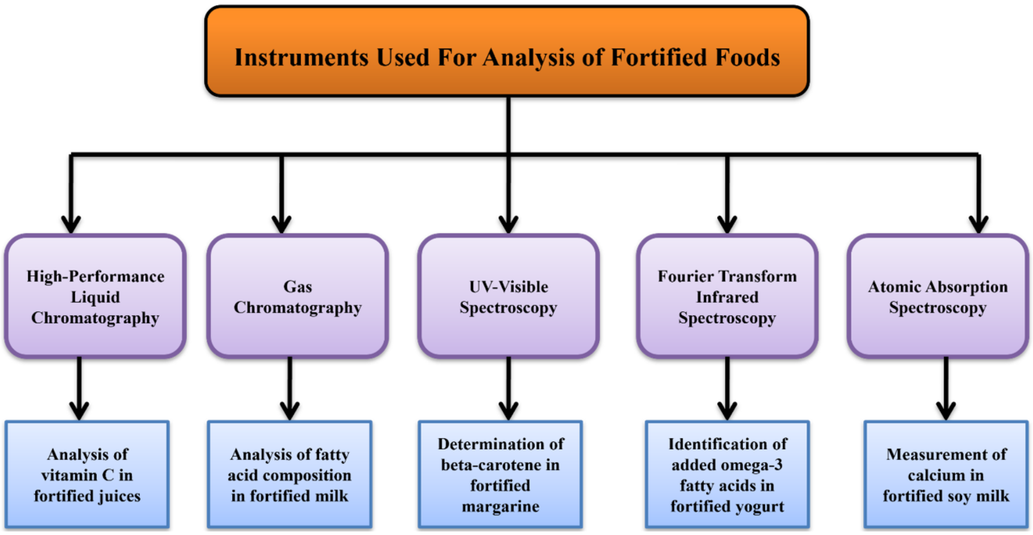

Figure 11.3: Analytical techniques and their application in the analysis of fortified foods (Benassi et, al).

11.3.1 Spectrophotometry:

Spectrophotometry is a versatile analytical technique that measures the absorption or transmission of light at specific wavelengths of a sample. In the context of nutritional analysis, UV-Vis spectrophotometry plays an essential role in determining vitamins such as A, E and C in fortified foods. For example, the concentration of ascorbic acid (vitamin C) in food samples can be accurately determined by UV-Vis spectrophotometry at 265 nm, which corresponds to the maximum absorption wavelength of ascorbic acid (Huang and Xu, 2021). Fluorescence spectrophotometry, another variation, offers better sensitivity by measuring the fluorescence emitted by specific vitamins, making it particularly useful for identifying vitamins at low concentrations (Gonzalez and Perez, 2016).

11.3.2 Chromatography:

Chromatographic methods, including high-performance liquid chromatography (HPLC) and gas chromatography (GC), are commonly used to separate and quantify vitamins and minerals. HPLC is particularly effective for water-soluble vitamins such as B-complex vitamins. The reversed-phase HPLC method with a C18 column allows the simultaneous separation and determination of several B vitamins. GC with flame ionization detection (FID) is used for fat-soluble vitamins such as vitamins A and D (Wang et al., 2018). Ultra-high-performance liquid chromatography (UHPLC) offers even better resolution and faster analysis times, making it suitable for complex food matrices (de la Fuente et al., 2017).

11.3.3 Mass Spectrometry:

Mass spectrometry (MS), particularly when combined with chromatographic techniques such as liquid chromatography (LC-MS) or gas chromatography (GC-MS), stands out for its exceptional sensitivity and specificity in the analysis of vitamins and minerals in fortified foods. This combination leverages the separation capabilities of chromatography with the detection prowess of mass spectrometry, enabling the accurate identification and quantification of multiple analytes within complex food matrices(Smith & Thomas, 2020).For instance, liquid chromatography-tandem mass spectrometry (LC-MS/MS) is widely used for the simultaneous determination of several vitamins in fortified foods.

This technique excels in providing high accuracy and sensitivity, even for trace amounts of vitamins. LC-MS/MS can separate vitamins based on their interaction with the chromatographic medium and then detect them with high specificity through mass spectrometric analysis(Halasz & Fingas, 2014).

Table 11.1: Comparative table summarizing different analytical techniques used for fortified food analysis, including their applications, advantages, and limitations:

Analytical Techniques	Applications	Advantages	Disadvantages	References
Spectrophotometry (UV-vis)	Vitamins A, E, C	Simple,cost-effective, high specificity	Limited to compounds with distinct absorbance spectra	(Huang & Xu, 2021)
Fluorescence Spectrophotometry	Low-concentration vitamins, trace analysis	High sensitivity, specific for fluorescent compounds	Requires fluorescent compounds, potential interference	(Gonzalez & Perez, 2016)
High-Performance Liquid Chromatography (HPLC)	Vitamins, antioxidants	High precision, suitable for complex mixtures	Expensive equipment requires skilled operation	(Miller & Miller, 2010)
Gas Chromatography (GC)	Fat-soluble vitamins, fatty acids	High resolution, suitable for volatile compounds	Requires derivatization for non-volatile compounds	(Miller & Miller, 2010)
Mass Spectrometry (MS)	Trace nutrients, contaminants	High sensitivity and specificity	High cost, complex data interpretation	(Zhu & Gao, 2020)

Analytical Techniques	**Applications**	**Advantages**	**Disadvantages**	**References**
Potentiometry (ISE)	Minerals (e.g., Na, K, Ca)	Direct measurement, high-precision	Limited to ion-selective electrodes available	(Hulanicki et al., 2002)
Voltammetry (ASV)	Heavy metals (e.g., Pb, Cd)	High sensitivity for tracemetals,relatively low cost	Requires preconcentration step, potential matrix effects	(Johnson et al., 2019; Sharma et al., 2016)

11.4 Electrochemical Methods:

Electrochemical methods, such as potentiometry and voltammetry, are key to the determination of essential minerals for nutritional analysis, including sodium, potassium and calcium. Potentiometry uses ion-selective electrodes (ISEs) to measure ion concentrations in food samples, providing a direct and accurate quantification based on the potential difference between the electrode and a reference electrode (Hulanicki et al., 2002). This method is very effective for estimating the mineral content of fortified foods, ensuring compliance with nutrition labelling and safety standards.Voltammetry encompasses various techniques, of which anodic stripping voltammetry (ASV) stands out for its ability to detect trace amounts of heavy metals such as lead and cadmium in food matrices (Zhu and Gao, 2020). ASV involves depositing an analyte on the electrode surface during electrolysis and then removing it by changing the potential, enabling sensitive quantification of heavy metal impurities at low concentrations. This method is crucial to control the concentration of heavy metals in fortified foods and to protect the health of the consumer by ensuring compliance with the permissible limits set by the authorities (Sharma et al., 2016). Both potentiometry and voltammetry offer clear advantages in mineral analysis due to their high sensitivity, selectivity and applicability in various food matrices. They enable accurate measurement of essential minerals and identification of impurities, supporting the food industry's quality control efforts to maintain nutritional integrity and safety.

Quality Control Measures:

Quality control is essential to ensure the accuracy and reliability of analytical results. Key quality control measures include the use of certified reference materials, method validation, and regular instrument calibration.

Certified Reference Materials (CRMs):

CRM is used to validate analytical methods and ensure traceability of measurement results. They act as benchmarks and help confirm the accuracy and reliability of analytical data. CRMs allow analysts to ensure that their methods produce consistent and accurate results (Taylor, 2018).

Method Validation:

Method validation is essential for evaluating the performance of analytical procedures by assessing key indicators such as accuracy, precision, specificity, sensitivity, and reproducibility. Parameters including the limit of detection (LOD), limit of quantification (LOQ), and linearity are critical to establish the method's suitability for intended applications (Brown et al., 2020).

Strong method validation ensures that analytical results are reliable and consistent, facilitating robust scientific conclusions and supporting regulatory compliance in food analysis.

Instrument Calibration:

Regular calibration of analytical instruments with standard solutions is essential to maintain accuracy and precision in food analysis. Calibration involves creating calibration curves by analyzing standard solutions of known analyte concentration and determining the relationship between instrument response and analyte concentration (Miller, 2017).

This practice ensures consistent device performance over time, enabling reliable quantification of nutrients and contaminants in fortified foods, supporting regulatory compliance and ensuring consumer safety.

11.5 Fortified Foods and Chemical Analysis:

Methods of chemical analysis of fortified foods have a multifaceted role that goes beyond mere adherence to official standards. These methods are necessary to improve the nutritional value of foods by accurately determining the added vitamins, minerals and other nutrients important for public health. By accurately measuring the level of fortification through analytical techniques such as spectrophotometry, chromatography and mass spectrometry, food manufacturers and regulatory agencies can ensure that fortified products meet recommended nutritional values and effectively address widespread nutritional deficiencies (Codex Alimentarius, 2021; Zimmermann 2007). In addition, chemical analysis plays a key role in assessing the stability and quality of fortified foods throughout their life cycle. This helps to control the degradation of added nutrients during processing, storage and distribution, ensuring that these products retain their nutritional value until consumption (FAO/WHO, 2018).

This aspect is crucial not only to maintain the effectiveness of nutritional supplement programs but also to ensure consumer confidence and satisfaction with the nutritional value advertised on food labels (Allen and de Benoist, 2006). In addition to nutritional content, these analytical methods are necessary for the detection and determination of impurities or unwanted additives that may threaten food safety. For example, heavy metals, pesticides or microbial contamination can accidentally enter food during production or packaging. Rigorous chemical analysis enables such hazards to be quickly identified and mitigated, ensuring compliance with public health and food safety standards (EFSA, 2020; FDA, 2021).In addition, chemical analyzes support accurate labelling of fortified foods, providing consumers with transparent information on nutritional content and facilitating informed food choices. By ensuring that nutritional claims on labels are consistent with analytical data, these methods enable consumers to make healthier decisions and promote overall nutritional balance and well-being (WHO, 2020). The various applications of chemical analysis in the evaluation of fortified foods include ensuring nutritional efficacy, monitoring product quality and safety, and providing transparent information to consumers. Using advanced analytical techniques, food industry stakeholders can maintain the integrity of nutritional supplementation programs, improve public health outcomes, and increase confidence in the nutritional value of fortified foods worldwide.

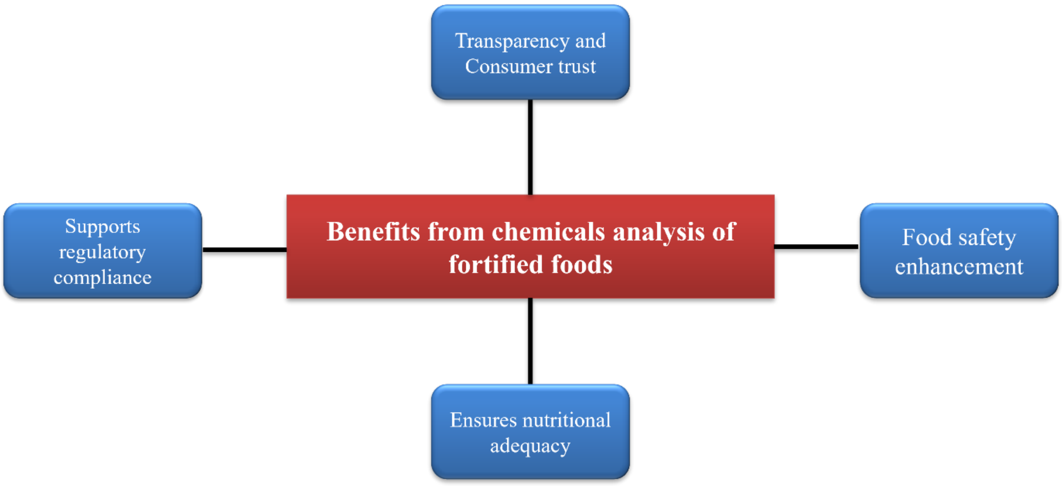

Figure 114: Benefits of chemical analysis of fortified foods. (Allen & de Benoist, 2006)

11.6 Conclusions:

In conclusion, to ensure the effectiveness, safety and quality of fortified foods, strict application of chemical analysis methods is essential. Accurate quantification of added nutrients such as vitamins and minerals is of utmost importance to meet regulatory standards and provide targeted health benefits to consumers (Allen and de Benoist, 2006). Advanced analytical techniques, including spectrophotometry, chromatography and mass spectrometry, provide the necessary sensitivity and specificity to accurately measure these nutrients even in small amounts (Miller and Miller, 2010; Smith and Thomas, 2020).

In addition, robust quality control measures such as the use of certified reference materials, method validation and regular calibration of instruments ensure the reliability and accuracy of analytical results (Thompson et al., 2004). These practices are necessary to maintain the nutritional integrity of fortified foods throughout their shelf life and to ensure that they remain safe and effective for consumers (FAO/WHO, 2018).Electrochemical methods such as potentiometry and voltammetry also play an essential role in mineral analysis, providing high accuracy and sensitivity for the identification of important minerals and potential contaminants (Johnson et al., 2019). The continued development and improvement of these analytical methods are critical to advancing nutritional supplementation programs and improving public health outcomes worldwide.

Finally, extensive application of chemical analysis methods in fortified foods not only supports regulatory compliance and food safety, but also provides consumers with reliable nutritional information, and promotes informed food choices and general well-being (FDA, 2021; WHO, 2020).

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